Breeding for Fruit Rot Resistance in *Vaccinium macrocarpon*

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Keywords: cranberry, *Phyllosticta vaccinii*, *Physalospora vaccinii*, *Glomerella cingulata*, *Colletotrichum gloeosporioides*

Abstract

The cranberry fruit rot complex can cause severe crop loss and requires multiple fungicide applications each year. To identify sources of fruit rot resistance, fungicides were withheld from our germplasm collection in 2003 and 2004 and the collection was rated for fruit rot. In Sept. 2003, 70% of the 562 plots had severe rot, while 6% showed some level of resistance. Visual ratings correlated with quantitative assessments. In 2004, several accessions continued to show resistance, and there was significant correlation between the 2003 and 2004 ratings (r=0.80) and counts (r=0.75). Three of the resistant accessions had previously been used in crosses and their progeny had been planted in a large progeny evaluation trial. Fungicides were withheld in 2005–2007 and the trial was rated each year for fruit rot. In 2007, disease pressure was so severe that of the 1644 progeny evaluated from 30 crosses (four of these crosses had a resistant parent), 1085 progeny exhibited nearly 100% rot, while only 13 plots had a rating of '2' (<40% fruit rot). Families from resistant parents had a higher frequency of resistant progeny, indicating additive genetic effects, and the potential for improving resistance through breeding. However, a few resistant progeny originated from susceptible parents suggesting non-additive variance for field fruit rot resistance also exists. Fruit cultured from susceptible and resistant plots had the same species of fruit rot fungi present (primarily *Phyllosticta vaccinii, Physalospora vaccinii* and *Colletotrichum gloeosporioides),* suggesting broad-based resistance. DNA fingerprinting of resistant accessions identified several distinct types, offering potentially different sources of genetic resistance. These fruit rot-resistant plants have now been used in 60 crosses. Molecular markers for resistance are being developed, which will allow for more efficient progeny screening.

INTRODUCTION

Fruit rot is one of the most serious diseases affecting cranberry (*Vaccinium macrocarpon* Ait.) production in the northeastern United States (Oudemans et al., 1998), and it is becoming more of a concern in Wisconsin (McManus, 1998). Without multiple fungicide applications each year, fruit loss in New Jersey can reach 100%. Fruit rot is caused by a dynamic complex of 10 to 15 fungal species, varying by year and location (Stiles and Oudemans, 1999). Fruit rot is divided into two categories, field rot and storage rot, with infection beginning either at early bloom, late bloom, or harvest, depending upon the fungal species. The most commonly isolated fungi include *Physalospora vaccinii* (Shear) Arx & E. Muller, *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz. (teleomorph *Glomerella cingulata* (Stoneman) Spaulding & H. Schrenk), *Phyllosticta vaccinii* Earle, *Coleophoma empetri* (Rostr.) Petr., and *Phomopsis vaccinii* Shear in Shear, N. Stevens, & H. Bain (Stiles and Oudemans, 1999; Oudemans et al., 1998).

Developing cranberry cultivars with enhanced resistance to the fruit rot disease complex would be an important contribution to cranberry culture, resulting in improved yields and reduced fungicide applications. Little breeding has been done specifically on

disease or insect resistance in cranberry, except for breeding for 'non-feeding preference' towards the blunt-nosed leafhopper which vectors the false blossom disease phytoplasma (Eck, 1990). Early breeding work used percent fruit rot as a selection criterion, and eliminated seedlings with severe fruit rot (Chandler et al., 1947). General descriptions of cranberry cultivars note differences in keeping quality, suggesting differences in resistance to storage rot fungi (Eck, 1990). When berries from 10 cranberry cultivars were cultured, no significant differences in fungal profiles were found, suggesting that resistance may be general rather than fungal species-specific (Stiles and Oudemans, 1999). Caruso and Mika (as reported in Oudemans et al., 1998) screened 44 cranberry cultivars and found differences in the incidence of field and storage rot fungi, indicating differences in resistance. Cranberry breeding trials in New Jersey routinely evaluate for percent fruit rot along with yield and fruit quality, and this data is considered when making selection decisions (Vorsa, unpublished data). However, because our breeding plots typically receive the standard regimen of fungicide applications in order to assess yield, the low fruit rot selection pressure does not offer the opportunity to identify high levels of resistance.

In this study, fungicides were withheld from a cranberry germplasm collection to provide extreme fruit rot pressure. The collection was then screened for fruit rot resistance; the resistant germplasm was DNA fingerprinted and genetic similarity of resistant selections was determined; and progeny from crosses with a fruit-rot resistant parent were evaluated.

MATERIALS AND METHODS

Germplasm Screening

In 1988 through 1994, a collection of germplasm was made from wild and cultivated cranberry bogs throughout the U.S., including NJ, NY, MA, DE, WV, PA, MI, and WI. This collection included major and minor cultivars, genetic variants that had developed in cultivated beds many decades old, and wild cranberries collected from a diverse range of habitats. In 1995, 600 of these accessions were planted in 2.25 m^2 plots in two 0.2 ha beds at the P.E. Marucci Center, Chatsworth, NJ. Alleys between plots were maintained with regular herbicide applications. The plots received standard maintenance practices (i.e., fertilization, pesticides, water harvest) and were evaluated yearly for yield and fruit quality. In 2003 and 2004, fungicide applications were withheld and the trial was evaluated for fruit rot.

On Sept. 22, 2003, severe fruit rot was observed. The plots were given a visual rating for fruit rot infection, using a 1 to 5 scale, where 1=no rot, and 5=all fruit severely rotten. Selected accessions spanning the range of fruit rot ratings were then harvested (34 plots, 2 samples of 930 cm^2/plot), and counts were made of rotten and sound fruit. In 2004, fruit was collected from 68 plots (930 cm^2 sample/plot) on July 24, Aug. 23 and Sept. 27 to determine percent rotten fruit, and track increase in fruit rot over the season. On Sept. 7, 2004, all 600 plots were rated for fruit rot.

In 2004, berries from four of the most susceptible accessions, and four of the most resistant accessions, were surface sterilized and plated on V8-juice agar medium to determine the species of fungi present and their prevalence. Approximately six rotten and eight sound berries were evaluated for each of the eight accessions on July 24, Aug. 23, and Sept. 27, 2004, for a total of over 300 berries.

DNA Fingerprinting

To determine the genetic similarity of the resistant accessions, DNA fingerprinting was done using the SCARs (Sequence Characterized Amplified Regions) method (Polashock & Vorsa, 2002). DNA was extracted from young leaves collected from the plots showing resistance, along with control samples. Each accession was scored for the presence (1) or absence (0) of all markers. The data were analyzed using NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System, Exeter Software, Setauket,

NY). SIMQUAL (NTSYS-pc) was used to determine similarity using the DICE (Dice, 1945) coefficient). The phenogram was produced by a UPGMA (unweighted pair-group method arithmetic average) clustering of the resulting (i.e. the SIMQUAL) matrix.

Progeny Screening

Three of the resistant or moderately resistant accessions, US89-3, US88-1 and US88-70, had been used in crosses in 1997 and 1998 (prior to being identified as resistant). Their progeny, along with progeny from susceptible parents, had been planted in a progeny evaluation trial in May 2000 at the Marucci Center (2.25 m^2 plots in a 2 ha bed). In 2005, 2006 and 2007, fungicides were withheld and the plots were rated each year for fruit rot using the 1 to 5 visual rating scale as noted above. Fruit was also collected and percent rotten fruit determined.

In September 2007, 190 berries were sampled from this trial and plated to determine fungal species present; berries were from five resistant and three susceptible progeny plots.

RESULTS AND DISCUSSION

Germplasm Screening

On Sept. 22, 2003, 70% of the accessions had severe rot (392 plots out of 562 had a rating of '5'), and only 6% showed some resistance (33 plots with a rating of '1' or '2') (Fig. 1). Fruit rot counts ranged from 5% to 100% rotten fruit (mean=56%). Ratings and counts were highly correlated (r=0.92), indicating that the visual ratings were a good estimate of percent rotten fruit.

On July 24, 2004, fruit rot was less prevalent because of the earlier sampling date (mean=8%), although some of the more susceptible selections already had 28% rotten fruit. By Sept. 27, mean percent rot was up to 66%; however, several accessions continued to have low fruit rot.

The distribution of ratings on Sept. 7, 2004 was similar to 2003 (Fig.1, Table 1), with the majority of accessions getting a rating of '5'. Ratings and counts in 2004 were again highly correlated (r=0.80); and when the two years of data were compared, ratings (r=0.80) and counts (r=0.75) were highly correlated. However, fruit rot counts are generally more accurate than visual assessment of field plots and sometimes provided better ranking of accessions with moderate ratings. For example, US88-70 had a mean rating of 3.5 but only 35% rotten fruit, and thus was considered moderately resistant.

When berries were cultured in July 2004, resistant selections had more berries that yielded no microbes, and *Phyllosticta vaccinii* (Early Rot) was the only pathogenic fungus present. Susceptible selections had more infected berries, and three pathogenic fungi were present, *Phyllosticta vaccinii, Physalospora vaccinii,* and *Colletotrichum gloeosporioides* (Fig. 2). Berries cultured in late August 2004, however, had the same species of fruit rot fungi present on the resistant and susceptible selections (Fig. 2), suggesting that the resistance is non-fungal species specific. In addition, since fruit rot fungi sometimes grew out of sound surface-sterilized fruit of the resistant selections when cultured, this suggests that the fungi are present in the berry, but only as latent infections. However, our sample size was relatively small given the variability typically found when isolating fruit rot pathogens. Additional sampling of resistant and susceptible selections throughout the season would be useful.

DNA Fingerprinting

DNA fingerprinting results are presented as a phenogram (Fig. 3). The Coefficient of Similarity (x-axis) indicates the relative similarity of the accessions tested, with those joined by a vertical line at 1.00 (100%) being identical (e.g., US94-22 and US88-107). The further to the left that the branches converge, the more genetically dissimilar they are. For example, US89-3 is only about 57% similar to US88-1 (or any of the 16 accessions in the upper cluster).

DNA fingerprinting of fruit rot-resistant accessions identified several genetically distinct-types, including 'Holliston-types' (US88-1, US88-68), 'Budd's Blues' (US88-30), a number of accessions with a 'Budd's Blues' phenotype (US94-176, US94-161), 'Cumberland' (US88-79), and US89-3 (Fig. 3, Table 1). 'Budd's Blues' had previously been recognized as having fruit rot resistance and is unique because of the heavy waxy bloom on the fruit (A.W. Stretch, pers. comm.). Unfortunately, it has very poor yield so is not commercially viable. In addition, 'Budd's Blues' progeny are generally not productive, although some can have moderate yields. 'Cumberland' (US88-79), on the other hand, typically has better yields. US89-3 is also of interest to us because previous studies found it to have high total phenolics. Phenolic compounds have been associated with disease resistance in other plants. The genetic dissimilarity found among the fruit rot-resistant accessions, as estimated by SCARs, suggests multiple sources of resistance, and an opportunity to make crosses among them to further enhance resistance.

Progeny Screening

On Aug. 31, 2005, 512 progeny plots from 11 crosses were rated for fruit rot (five crosses had a resistant parent). Fruit rot was severe, with 342 of the plots (66%) having a rating of '4' or '5', and only 28 plots (5%) having a rating of '1' or '2'. In 2006, the same progeny were rated on Aug. 21. Because of the earlier evaluation date, fruit rot was less severe; 133 plots had a rating of '4' or '5' (26%), and 74 plots had a rating of '1' or '2' (15%). In 2007, an additional section of the trial was left untreated with fungicides so a total of 1644 plots from 30 crosses were rated for fruit rot on Aug. 20. Disease pressure was so severe that 1085 plots had a rating of '5' (66%), while only 13 plots had a rating of '2' (0.8%). Ratings between years were significantly correlated and fruit rot counts were correlated with ratings.

Family frequency distributions for rot ratings indicated families with a resistant parent had a higher frequency of resistant progeny. For example, if two crosses using a common susceptible cultivar 'Stevens' are compared ('Stevens' × resistant accession US88-70, and 'Stevens' × susceptible US88-81), the cross with resistant US88-70 exhibits a higher frequency of progeny with low ratings (Fig. 4). This observation was consistent all three years, indicating the potential for improving fruit rot resistance. However, a few resistant progeny also originated from susceptible parents, suggesting non-additive variance effects. It is likely that multiple loci are involved in resistance and susceptible plants can carry alleles for resistance. Additional testcrosses and evaluations are needed to determine inheritance of resistance.

These plots and data will be further evaluated in 2008. Because several of the fruit rot fungi infect the berry very early in its development, the flowering phenology of resistant and susceptible progeny will be compared.

Fruit cultured from this trial in 2007 had a similar species profile of fruit rot fungi present on the resistant and susceptible selections, primarily *Phyllosticta vaccinii, Physalospora vaccinii* and *Colletotrichum gloeosporioides*, further supporting the hypothesis of broad-based resistance.

Identifying fruit rot-resistant cranberry germplasm is just a first step in developing resistant cultivars. Once identified, the resistance must be incorporated into a high-yielding, high-quality cranberry. The fruit rot-resistant plants identified in this study have now been used in over 60 crosses with our most elite selections. We will begin evaluating several thousand of these progeny in 2009. Our results indicate multiple sources of fruit rot resistance, and suggest that the resistance is non-fungal species specific and multigenic. Variation appears to have an additive and a non-additive component. Development of molecular markers for resistance is underway, which may allow more efficient progeny screening.

ACKNOWLEDGEMENT

The authors thank Chris Constantelos for assistance with culturing and fungal identification, Kristia Adams for technical assistance with DNA fingerprinting, and Karen

DeStefano, Susan Vancho and Leah Ruban for technical assistance. This research was partially funded by Ocean Spray Cranberries, Inc., USDA CSREES, and NJAES Hatch.

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Tables

Table 1. Fruit rot ratings of cultivars and selections in a germplasm evaluation trial planted in 1995 at Chatsworth, NJ.

Cultivar or Selection	Code*	Fruit rot rating (1=no rot, 5=100% rot)		
		22-Sep-03	7-Sep-04	mean
Drever	US88-1	1.0	1.0	1.0
Haines Blues-1	US94-176	1.0	1.0	1.0
Haines Blues-2	US94-181	1.0	1.0	1.0
Budd's Blues	US88-30	1.0	1.0	1.0
Budd's Blues-Type	US93-34	1.0	1.0	1.0
Champion	US88-116	2.0	1.0	1.5
Cumberland	US88-79	2.0	1.0	1.5
Holliston-Type	US88-68	2.0	1.0	1.5
Paradise Meadow-1	US88-97	1.0	3.0	2.0
US88-121	US88-121	2.0	2.0	2.0
US89-3	US89-3	2.0	2.0	2.0
Paradise Meadow-2	US88-85	3.0	2.0	2.5
Grygleski Hybrid #3	US94-6	3.0	2.0	2.5
Wales Henry	US88-67	2.0	3.0	2.5
AR2	US88-43	2.0	3.0	2.5
Cutts Bog Tetpld B	US94-57	3.0	2.0	2.5
US94-93	US94-93	3.0	2.0	2.5
Gebhardt's Beauty	US88-115	2.0	3.0	2.5
US94-12	US94-12	2.0	3.0	2.5
Holliston-Type	US88-59	3.0	3.0	3.0
Grygleski Hybrid #2	US94-5	3.0	3.0	3.0
Pilgrim Lake, Mass	NJ91-13-7	3.0	3.0	3.0
Wi Tetraploid B	US94-67	3.0	3.0	3.0
Hollister Red	US88-70	3.0	4.0	3.5
Lemunyon		3.8	3.9	3.9
Franklin		4.0	4.0	4.0
Wilcox		4.0	4.5	4.3
#35		4.5	4.5	4.5
Pilgrim		4.0	5.0	4.5
Early Black		4.5	4.6	4.6
Potter		4.6	4.6	4.6
Stevens		4.8	4.5	4.6
Bergman		4.3	5.0	4.7
Searles		4.8	4.6	4.7
Shaw's Success		5.0	4.5	4.8
Cropper		4.6	5.0	4.8
Howes		4.8	4.9	4.8
Mcfarlin		5.0	4.8	4.9
Aviator		5.0	5.0	5.0
Ben Lear		-	5.0	5.0
Black Veil		5.0	5.0	5.0
Early Richard		5.0	5.0	5.0
Mean of 562 accessions		4.5	4.5	4.5

*Code is the designation given to each accession when collected in 1988–1994. Cultivars without codes are the means of multiple plots of that cultivar (means taken only when plots were identical by DNA fingerprinting).

Figures

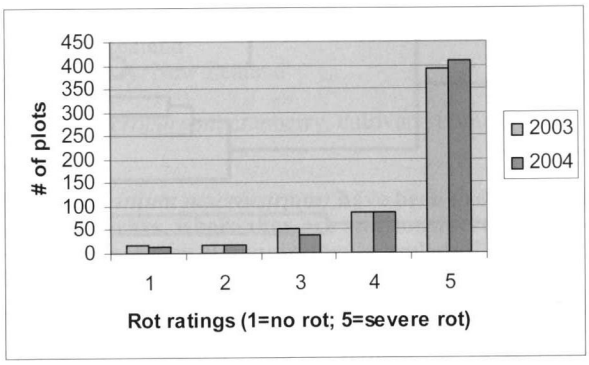

Fig. 1. Distribution of fruit rot ratings in a germplasm collection, 9/22/03 and 9/7/04.

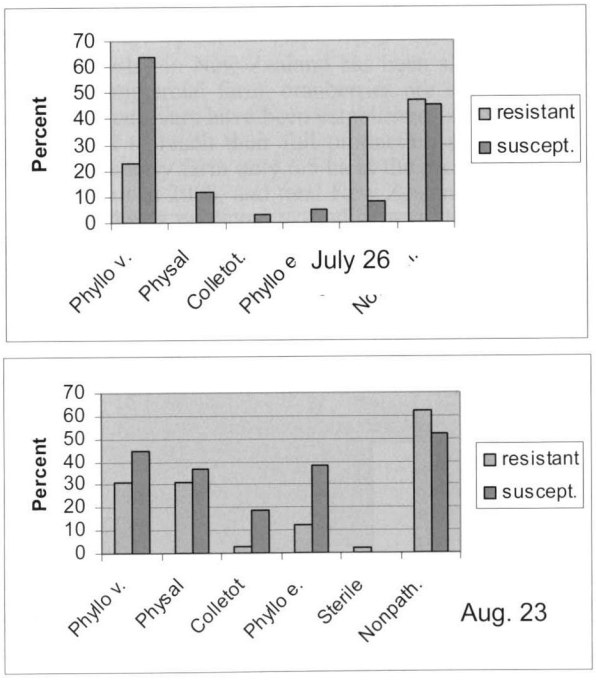

Fig. 2. Fungi found on resistant vs. susceptible accessions on July 26 and Aug. 23, 2004.

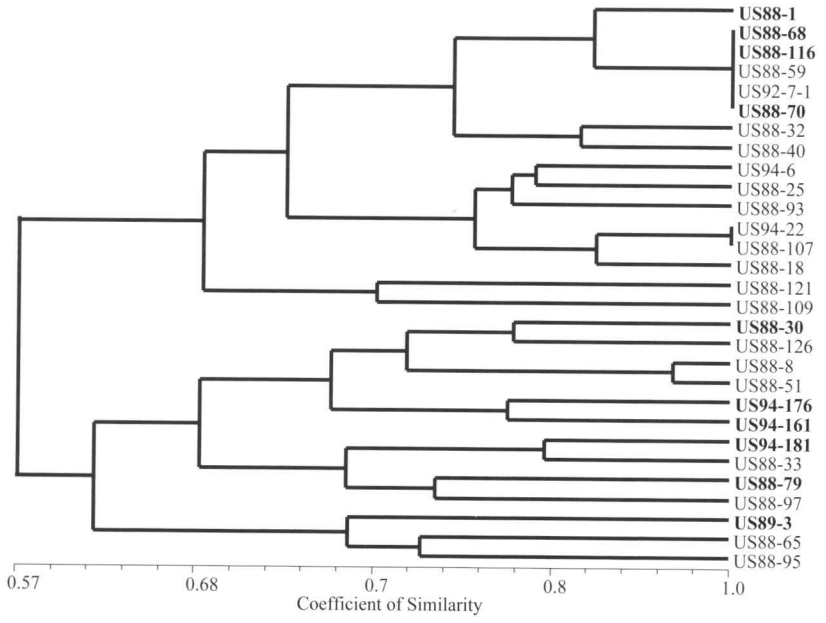

Fig. 3. Phenogram illustrating the genetic diversity of fruit rot-resistant accessions (resistant accessions indicated with **bold font**, see Table 1).

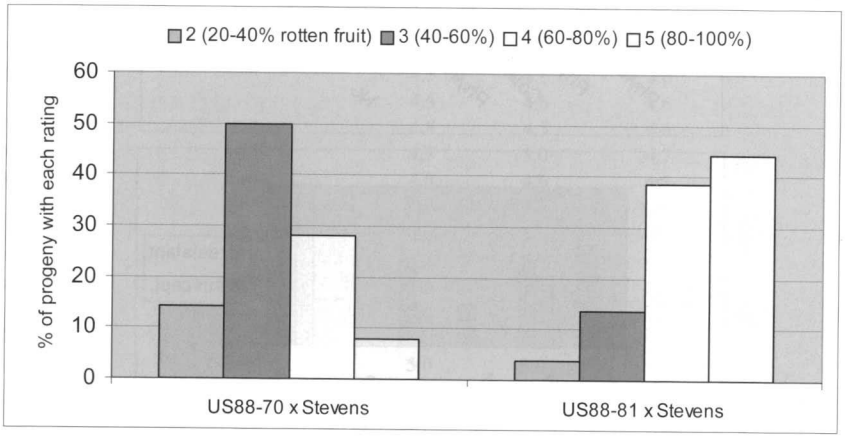

Fig. 4. Distribution of fruit rot ratings in two families, Stevens crossed with moderately resistant accession US88-70 vs. Stevens crossed with susceptible accession US88-81; progeny rated on Aug. 31, 2005 using a 1-5 visual rating scale.